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(54) Title: POLYPEPTIDES OF LIPOPOLYSACCHARIDE BINDING PROTEIN			
(57) Abstract			
<p>The present invention provides a first polypeptide fragment of lipopolysaccharide (LPS) binding protein (LBP) which binds to lipopolysaccharide, but prevents the LPS:LBP complex from either transferring LPS to CD14 or promoting the formation of an LPS:CD14 complex and a second polypeptide fragment of LBP which binds to CD14 receptor to inhibit binding of LPS:LBP complex to the CD14 receptor. Also included are methods of ameliorating symptoms of sepsis in a subject by administration of an LBP polypeptide of the invention, or administration of antibody to LBP polypeptide or anti-idiotype antibody.</p>			

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POLYPEPTIDES OF LIPOPOLYSACCHARIDE BINDING PROTEIN

This invention was made with Government support under Grant No. AI 25563, AI 32021 and AI 15136 awarded by the National Institute of Health. The Government has certain rights in this invention.

5 BACKGROUND OF THE INVENTION

1. *Field of the Invention*

The present invention relates generally to polypeptides of lipopolysaccharide binding protein (LBP) that inhibit the binding of lipopolysaccharide (LPS) released by gram-negative bacteria to the CD14 receptor, and specifically to the use of these polypeptides to ameliorating sepsis and the symptoms of sepsis in a subject and for assaying for gram-negative bacterial LPS.

2. *Description of Related Art*

Sepsis is induced by a toxin, the introduction or accumulation of which is most commonly caused by infection or trauma. The initial symptoms of sepsis typically include chills, profuse sweat, irregularly remittent fever, prostration and the like, followed by persistant fever, hypotension leading to shock, neutropenia, leukopenia, disseminated intravascular coagulation, adult respiratory distress syndrome and multiple organ failure.

Sepsis-inducing toxins have been found associated with pathogenic bacteria, viruses, plants and venoms. Among the well described bacterial toxins are the endotoxins or lipopolysaccharides (LPS) of the gram-negative bacteria. These molecules are glycolipids that are ubiquitous in the outer membrane of all gram-negative bacteria. The gram-negative bacteria of the gastrointestinal tract produce disease by invasion of tissue and by release of pharmacologically active LPS from the cell wall. Endotoxins from a wide variety of unrelated species behave quite similarly, regardless of the inherent pathogenic-

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ity of the microorganism from which they are derived or their antigenic structure. In the intact microorganism, endotoxins exist as complexes of lipid, polysaccharide, glycolipid and non-covalently-bound protein. The biologic activity seems to be a property of a lipid and carbohydrate portion.

5 Septic shock is characterized by inadequate tissue perfusion, most frequently following gram-negative bacteremia. The most common causative organisms are *Escherichia coli*, *Klebsiella-Enterobacter*, *Proteus*, *Pseudomonas*, and *Serratia*. *Neisseria meningitidis* bacteremia and gram-negative anaerobic bacteremia with *Bacteroides* spp are also important causes of septic shock. Most of the bacteria which cause gram-negative sepsis
10 are normal commensals in the gastrointestinal tract. From there they may spread to contiguous structures, as in peritonitis after appendiceal perforation, or they may migrate from the perineum into the urethra or bladder.

15 The primary response of the host to LPS involves the recognition of LPS by cells of the monocyte/macrophage lineage, followed by the rapid elaboration of a variety of cell products including the general group known as cytokines. Other cell types believed to participate in sepsis and in particular in the response to LPS are polymorphonuclear leukocytes and endothelial cells. Each of these cell types are also capable of responding to LPS with an elaboration of potent inflammatory substances.

20 LPS is believed to be a primary cause of death in humans during gram-negative sepsis, particularly when the symptoms include adult respiratory distress syndrome (ARDS). One particular cytokine, tumor necrosis factor (TNF), has recently been reported to be a primary mediator of septic shock (Beutler, *et al.*, *New Eng. J. Med.*, 316:379, 1987). Intravenous injection of LPS endotoxin from bacteria into experimental animals and man produces a rapid, transient release of TNF (Beutler, *et al.*, *J. Immunol.*, 135:3972, 1985).
25 Evidence that TNF is a critical mediator of septic shock comes primarily from experiments in which pretreatment of animals with anti-TNF antibodies reduces lethality (Beutler, *et al.*, *Science*, 229:869, 1985; Mathison, *et al.*, *J. Clin. Invest.* 81:1925, 1988).

These reports suggest that inhibition of the secretion of TNF caused by LPS or other factors would ameliorate the often lethal symptoms of sepsis.

LPS binding protein (LBP) is a 58-60 kD serum glycoprotein which participates in the LPS-dependent activation of myeloid, endothelial, and epithelial cells. It does so by first 5 binding to LPS to form a high affinity LPS:LBP complex (Schumann, *et al.*, *Science*, 249:1429, 1990; Tobias, *et al.*, *Am. J. Respir. Cell. Mol. Biol.* 1:239, 1992). The complex then interacts with CD14 to form a LBP:LPS:CD14 complex. CD14 is present *in vivo* in two forms. Myeloid cells express a glycerophosphorylinositol-tailed, membrane-bound 10 form of CD14 (mCD14). Binding of LPS to mCD14 is promoted by LBP and results in cell activation (Tobias, *et al.*, *J. Immunol.* 150:3011, 1993; Ulevitch and Tobias, *Curr. Opin. Immunol.*, 6:125, 1993). Additionally, a soluble form of CD14 without the glycerophosphorylinositol-tail (sCD14) circulates in the plasma. LBP also promotes the formation of LPS:sCD14 complexes. The LPS:sCD14 complexes then react with as yet 15 unidentified receptors on epithelial cells resulting in cell activation (Frey, *et al.*, *J. Exp. Med.*, 176:1665, 1992; Pugin, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:2744, 1993). Thus, it appears that LBP has at least two functions, formation of an LPS:LBP complex and promotion of the formation of an LPS:CD14 complex.

It is desirable to inhibit LPS:LBP:CD14 complex formation or inhibit the LPS:LBP complex from transferring LPS to CD14 to form an LPS:CD14 complex. The present 20 invention provides polypeptides of LBP which bind to LPS, but prevents the LPS:LBP complex from promoting the formation of an LPS:CD14 complex and prevents LPS transfer to CD14 and also polypeptides of LBP which inhibit the binding of LPS:LBP complex to CD14.

SUMMARY OF THE INVENTION

The present invention is based on the unexpected discovery that a first specific region of the lipopolysaccharide (LPS) binding protein (LBP) is involved in binding to LPS, but lacks the ability to promote the formation of a LPS:LBP:CD14 complex. Thus, the 5 present invention provides a first polypeptide of LBP which, like native LBP, retains the ability to bind to LPS, but, unlike native LBP, does not have the ability to promote the formation of an LPS:CD14 complex.

The invention also provides a second specific region of LBP which, in contrast to the first 10 polypeptides, does not bind to LPS, but binds to CD14. Thus, this second polypeptide of LBP can inhibit the interaction of LPS:LBP complex with CD14.

In the first embodiment of the invention, the amino acid sequence of the polypeptides of LBP is provided. Due to its ability to bind and form a complex with LPS, the first polypeptide of LBP is useful in an assay to detect LPS endotoxin in a sample.

The invention also provides a method of ameliorating sepsis or the symptoms of sepsis 15 in a subject, comprising administering a therapeutically effective amount of polypeptide of LBP or antibody to the polypeptide of LBP. In addition, an antibiotic, anti-tumor necrosis factor (TNF) antibody or both, can be administered to the subject.

Finally, the invention provides a therapeutic composition comprising a polypeptide of LPS binding protein which inhibits the binding of an LPS:LBP complex to CD14.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows an SDS-PAGE analysis of the purification of the amino terminal amino acids of LBP (NH-LBP). The left panel is a Coomassie-stained gel. The right panel shows a Western blot of an equivalent gel using polyclonal goat antiserum to human LBP.

5 Lanes 1 and 5 show supernatant from untransfected cells; lanes 2 and 6 show supernatant from NH-LBP transfected cells; lanes 3 and 7 show NH-LBP partially purified by Bio-Rex 70 chromatography; and lanes 4 and 8 show human LBP.

FIGURE 2 shows a functional analysis of NH-LBP using ^{125}I -ASD-LPS (2-(p-azidosalicylamido) ethyl-1,3'-dithiopropionate) labeling. Reaction mixtures contained LBP at 0.5×10^{-8} M and/or sCD14 at 9×10^{-8} M as indicated by (+). The concentrations of NH-LBP indicated in the figure are in units of 10^{-8} M. For lanes 1-9, all components were mixed with the ^{125}I -ASD-LPS added last. For lanes 10-12, the ^{125}I -ASD-LPS and NH-LBP were incubated for 10 min at room temperature before addition of the LBP and sCD14.

15 FIGURE 3A shows FACS analysis of FITC-LPS binding to hCD14-CHO cells. 1, cells alone; 2, FITC-LPS plus NH-LBP with cells; 3, FITC-LPS plus LBP with cells; 4, FITC-LPS plus NH-LBP plus LBP with cells.

FIGURE 3B shows inhibition of FITC-LPS binding to hCD14-CHO cells by NH-LBP. hCD14-CHO cells were mixed with FITC-LPS (5 ng/ml) in the presence of the indicated 20 concentrations of NH-LBP with (□) or without (○) LBP (100 ng/ml).

FIGURE 4 shows the inhibition of LPS-initiated rabbit PEM activation by NH-LBP. Results are shown as TNF (Units/ml) for LBP and LBP+NH-LBP.

FIGURES 5a, b, and c show the nucleotide and deduced amino acid sequence of human LBP (SEQ ID NO: 8).

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FIGURE 6 shows the nucleotide and deduced amino acid sequence of amino acid residues 1-197 of human LBP (SEQ ID NO: 1 and 2).

FIGURES 7a and b show the nucleotide and deduced amino acid sequence of amino acid residues 198-481 of human LBP (SEQ ID NO: 6 and 7).

DETAILED DESCRIPTION OF THE INVENTION

The formation of a complex of lipopolysaccharide (LPS) and a full length lipopolysaccharide binding protein (LBP) polypeptide promotes the interaction of the LPS complex with either the soluble form of cell surface marker CD14 (sCD14) or membrane bound CD14 (mCD14). LPS:LBP complexes activate mononuclear blood cells by binding to the mCD14, triggering the production of cytokines for activation of endothelial cells. The present invention provides a "first polypeptide of LBP" or "first LBP polypeptide" which binds to LPS, but does not retain the ability to promote the association of a LPS with CD14, since the first polypeptide lacks that region of the LBP which has been now identified as being responsible for binding of the LPS:LBP complex to CD14. The polypeptide region of native or full-length LBP which is responsible for interaction with CD14 is noted herein as the "second polypeptide of LBP" or "second LBP polypeptide".

In a first embodiment, the invention provides an isolated first polypeptide of LBP with an amino acid sequence of SEQ ID NO:2, a second polypeptide with an amino acid sequence of SEQ ID NO:7 and functional fragments of the first and second polypeptides. The term "isolated" as used herein refers to polypeptide of LBP which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify polypeptide of LBP using standard techniques for protein purification. The substantially pure first polypeptide will yield a single major band of about 27,000 daltons on a non-reducing polyacrylamide gel, whereas the second polypeptide has a single major band of about 31,000 daltons on a non-reducing polyacrylamide gel.

The first and second LBP polypeptide of the invention include "functional fragments" of the polypeptide, as long as the activity of the LBP polypeptide remains. Smaller peptides containing the biological activity of LBP polypeptides specifically exemplified herein are included in the invention.

5 The first LBP polypeptide of the invention refers to a polypeptide having the amino acid sequence of SEQ ID NO:2 and consists of amino terminal residues 1-197 of the native LBP. The present invention has identified the amino terminal region of LBP as having the LPS binding region, while the remaining carboxy terminal region (also noted herein as the second LBP polypeptide of the invention) is responsible for either transferring LPS to sCD14 or for forming a complex between LBP:LPS and CD14. Therefore, functional 10 fragments of SEQ ID NO:2 include those amino terminal fragments which retain the ability to bind to LPS and which prevent LPS from associating with native LBP. An assay for determining whether a particular fragment of interest retains the functional activity of the polypeptide of SEQ ID NO: 2 is described in Example 2 of the present 15 application. Briefly, functional fragments of SEQ ID NO:7 include those carboxy terminal fragments which retain the ability to inhibit the transfer of LPS to sCD14 or to inhibit the interaction or association of LPS:LBP complex with CD14.

20 One of skill in the art is able to determine whether a particular fragment of interest has the functional activity of the polypeptide of the invention. For example, the assay outlined in Example 2 could be used to determine whether a polypeptide of interest has the ability to bind to LPS, but not CD14, or whether a polypeptide blocks LBP-LPS interaction. The ability of an amino terminal polypeptide of LBP to inhibit fluorescein-labeled LPS (FITC-LPS) binding to CD14 expressing cells can easily be assessed by FACS analysis. A second assay described in Example 2 utilizes a photoactivatable derivative of LPS which, 25 upon photolysis, radioiodinates proteins to which it binds. Other labels and methods of assaying for functional equivalents of the polypeptides of the invention will be known to those of skill in the art.

The invention also provides polynucleotides encoding the LBP polypeptides of the invention. These polynucleotides include DNA, cDNA and RNA sequences which encode the LBP polypeptide. Therefore, the sequence as shown in SEQ ID NO: 1 and 6, also, includes those sequences where T (thymidine) is U (uracil) and nucleic acid sequences complementary to the sequence ID's shown herein. It is understood that all polynucleotides encoding all or a portion of LBP polypeptide are also included herein, as long as they encode a polypeptide with the activity of first LBP polypeptide or second LBP polypeptide, e.g., bind to LPS. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, LBP polypeptide polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for LBP polypeptide also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of the LBP polypeptide encoded by the nucleotide sequence is functionally unchanged.

Minor modifications of the recombinant LBP polypeptide primary amino acid sequence may result in polypeptides which have substantially equivalent activity as compared to the first or second LBP polypeptides described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of first or second LBP polypeptide still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which will not affect or are not required for LBP polypeptide biological activity.

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The nucleotide sequence encoding the LBP polypeptides of the invention includes the disclosed sequences and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution 5 of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted 10 polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences and 2) antibody screening of 15 expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the LBP polypeptide polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. 20 Oligonucleotide probes, which correspond to a part of the sequence encoding the polypeptide in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction 25 when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an

extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that 5 single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981; Ausubel, *et al.*, *ed.*, *Current Protocols in Molecular Biology*, 1989).

The development of specific DNA sequences encoding LBP polypeptide of the invention can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary 10 codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA. In addition, the LBP polypeptides of the invention can be obtained by polymerase chain reaction (PCR).

15 Of the above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

20 The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived 25 from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant

portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been 5 denatured into a single-stranded form (Jay, *et al.*, *Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for LBP polypeptide having at least one epitope, using antibodies specific for the LBP polypeptide. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of LBP polypeptide cDNA.

10 DNA sequences encoding LBP polypeptide of the invention can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are 15 included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the LBP polypeptide polynucleotide sequences may be inserted 20 into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the LBP genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed 25 cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression

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in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding LBP polypeptides of the invention can be expressed

5 in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

10 Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

15 When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the LBP polypeptides of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example,

20 25 *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

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Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

5 The invention includes antibodies which bind to LBP polypeptides or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 10 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')₂, which are capable of binding an epitopic determinant on LBP polypeptide. An antibody to LBP polypeptide of the invention would bind within the amino terminal sequence of first LBP polypeptide and prevent LPS from forming a complex with LBP or from forming a complex with CD14. 15 Therefore, the antibody to first LBP polypeptide competitively inhibits the binding of LPS binding protein to LPS or LPS:LBP binding protein complex to CD14.

Likewise, antibody to the second LBP polypeptide of the invention would inhibit the binding of LPS:LBP complex to CD14 by blocking the interaction between the CD14 binding region of native LBP and CD14.

20 It is also possible to use anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody. Thus, in the present invention, an anti-idiotype antibody produced from an antibody which binds to a LBP polypeptide as in SEQ ID NO:2, or a synthetic peptide of SEQ ID NO:2, can act as a 25 competitive inhibitor for a site on full length, native LBP which is required for binding to LPS, thereby preventing LPS from forming a complex with or being transferred to

CD14 and thereby preventing activation of monocytes and other cells. Alternatively, an anti-idiotype antibody produced from an antibody which binds to an LBP polypeptide as in SEQ ID NO:7, or a synthetic peptide of SEQ ID NO:7 can act as a competitive inhibitor for a site on full length, native LBP which is required for binding of LPS:LBP complex with CD14.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier.

10 In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention 15 can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. A technique which may result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which 20 reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific antihapten antibodies. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

25 The presence of LPS endotoxin secreted by gram-negative bacteria, for example, can be detected *in vitro* in a liquid body sample or other aqueous body sample that is suspected of containing LPS. Exemplary body samples include blood, serum, plasma, saliva, urine, and cerebrospinal fluid. Blood, serum and plasma are preferred body samples.

The body sample suspected of containing LPS is admixed with an LBP polypeptide as described to form an admixture. In the case of LBP first polypeptide, the admixture is maintained for an amount of time sufficient for the LBP polypeptide to react and form a complex with the LPS endotoxin present in the sample, for example about 10 minutes.

5 It is well known in the art that the incubation time is a function of the amount of both the LBP and LPS in the admixture, with lower amounts typically requiring longer incubation times. Therefore, about 5 minutes to about 3 hours and preferably about 10 minutes to about 30 minutes is typical. The presence of the complex formed between the admixed first LBP polypeptide and LPS endotoxin is determined. The first LBP polypeptide is
10 preferably labeled with a means for indicating the formation of the complex and the amount of complex formed.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, 15 chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Enzyme labels and their substrates include horseradish peroxidase and hydrogen peroxide and an oxidative dye precursor such as o-phenylenediamine and alkaline phosphatase typically used with p-nitrophenyl phosphate. Exemplary radioisotopes include ³H and ¹²⁵I. Those of ordinary skill in the art will know of other 20 suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Useful solid matrices which can be used in an *in vitro* detection method of the invention include such materials as cross-linked dextran (SEPHADEX), agarose, glass beads, nitrocellulose, or the wells of a microtiter plate such as those made from polystyrene or 25 polyvinyl chloride.

In using a first LBP polypeptide of the invention for the *in vivo* detection of LPS, the detectably labeled LBP is given a dose which is diagnostically effective. The term

"diagnostically effective" means that the amount of detectably labeled LBP polypeptide is administered in sufficient quantity to enable detection of the site having LPS.

In addition, a monoclonal antibody that binds to first or second LBP polypeptide could be used to detect LBP:LPS complexes in a subject.

- 5 The concentration of detectably labeled monoclonal antibody or first LBP polypeptide which is administered should be sufficient such that the binding to those cells having LPS or soluble LPS is detectable compared to the background. Further, it is desirable that the detectably labeled antibody or first LBP polypeptide be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.
- 10 As a rule, the dosage of detectably labeled monoclonal antibody or first LBP polypeptide for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, LPS or antigenic burden, and other factors known to those of skill in the art.
- 15 For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.
- 20 For *in vivo* diagnosis radioisotopes may be bound to the antibody or first LBP polypeptide either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylene-

- 25 For *in vivo* diagnosis radioisotopes may be bound to the antibody or first LBP polypeptide either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylene-

triaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the antibodies or polypeptides of the invention are ¹¹¹In, ⁹⁷Ru, ⁶⁷Ga, ⁶⁸Ga, ⁷⁵As, ⁸⁹Zr, and ²⁰¹Tl.

5 The first LBP polypeptide or antibody to first or second LBP polypeptide of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include
10 ¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Cr, and ⁵⁶Fe.

15 The invention provides a method of ameliorating sepsis or one or more of the symptoms of sepsis comprising administering to a subject displaying symptoms of sepsis or at risk for developing sepsis, a therapeutically effective amount of first or second LBP polypeptide or antibody that binds to first or second LBP polypeptide. Such symptoms which may be ameliorated include those associated with a transient increase in the blood level of TNF, such as fever, hypotension, neutropenia, leukopenia, thrombocytopenia, disseminated intravascular coagulation, adult respiratory distress syndrome, shock and multiple organ failure. Patients who require such treatment include those at risk for or those suffering from toxemia, such as endotoxemia resulting from a gram-negative
20 bacterial infection, venom poisoning, or hepatic failure, for example. In addition, patients having a gram-positive bacterial, viral or fungal infection may display symptoms of sepsis and may benefit from such a therapeutic method as described herein. Those patients who are more particularly able to benefit from the method of the invention are those suffering from infection by *E. coli*, *Haemophilus influenza* B, *Neisseria meningitidis*,
25 *staphylococci*, or *pneumococci*. Patients at risk for sepsis include those suffering from burns, gunshot wounds, renal or hepatic failure.

The term "therapeutically effective amount" as used herein refers to the amount of either first or second LBP polypeptide, antibody to first or second LBP polypeptide or anti-idiotype antibody which binds a paratope of an antibody which binds to the amino acid sequence of first or second LBP polypeptide, such as in SEQ ID NO:2 or SEQ ID NO:7, 5 used in sufficient quantity to decrease the subject's response to LPS and decrease the symptoms of sepsis. The term "therapeutically effective" therefore includes that amount of first or second LBP polypeptide, antibody to first or second LBP polypeptide or anti-idiotype antibody to such antibody sufficient to prevent, and preferably reduce by at least 50%, and more preferably sufficient to reduce by 90%, a clinically significant increase 10 in the plasma level of TNF. The dosage ranges for the administration of the first or second LBP polypeptide, antibody to first or second LBP polypeptide antibody, or anti-idiotype antibody to such antibody of the invention are those large enough to produce the desired effect. Generally, the dosage will vary with the age, condition, sex, and extent of the infection with bacteria or other agent as described above, in the patient and can be 15 determined by one skilled in the art. The dosage can be adjusted by the individual physician in the event of any contraindications. In any event, the effectiveness of treatment can be determined by monitoring the level of LPS and TNF in a patient. A decrease in serum LPS and TNF levels should correlate with recovery of the patient.

In addition, patients at risk for or exhibiting the symptoms of sepsis can be treated by the 20 method as described above, further comprising administering, substantially simultaneously with the therapeutic administration of a first or second LBP polypeptide, antibody to first or second LBP polypeptide, or anti-idiotype antibody to such antibody, an inhibitor of TNF, an antibiotic, or both. For example, intervention in the role of TNF in sepsis, either directly or indirectly, such as by use of an anti-TNF antibody and/or a 25 TNF antagonist, can prevent or ameliorate the symptoms of sepsis. Particularly preferred is the use of an anti-TNF antibody as an active ingredient, such as a monoclonal antibody with TNF specificity as described by Tracey, *et al.* (*Nature*, 330:662, 1987).

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A patient who exhibits the symptoms of sepsis may be treated with an antibiotic in addition to the treatment with a first or second LBP polypeptide or antibody of the invention. Typical antibiotics include an aminoglycoside, such as gentamycin or a beta-lactam such as penicillin, or cephalosporin. Therefore, a preferred therapeutic method of 5 the invention includes administering a therapeutically effective amount of first or second LBP polypeptide, antibody to first or second LBP polypeptide, or anti-idiotype antibody to such antibody, substantially simultaneously with administration of a bactericidal amount of an antibiotic.

10 The term "bactericidal amount" as used herein refers to an amount sufficient to achieve a bacteria-killing blood concentration in the patient receiving the treatment. The bactericidal amount of antibiotic generally recognized as safe for administration to a human is well known in the art, and as is known in the art, varies with the specific antibiotic and the type of bacterial infection being treated.

15 Preferably, administration of a first or second LBP polypeptide, or antibody to first or second LBP polypeptide, including anti-idiotype antibody of the invention, occurs within about 48 hours and preferably within about 2-8 hours, and most preferably, substantially concurrently with administration of the antibiotic.

20 The method of the invention also envisions treating the patient with a combination of the above described therapies. In other words, a patient may be administered in various combination, first or second LBP polypeptide, or antibody to first or second LBP polypeptide, including anti-idiotype antibody of the invention, an appropriate antibiotic, and an agent which decreases TNF in the patient, such as anti-TNF antibody.

25 In another embodiment, the invention provides a therapeutic composition which includes in a pharmaceutically acceptable carrier, one or more of a first or second LBP polypeptide, antibody which binds first or second LBP polypeptide, or anti-idiotype antibody which binds a paratope of an antibody which binds to the amino acid sequence

of a first or second LBP polypeptide, such as in SEQ ID NO:2 or SEQ ID NO:7, respectively. As used herein, the term "pharmaceutically acceptable carrier" means a composition that is physiologically tolerable and does not typically cause an allergic or similar reaction, such as gastric upset or dizziness when administered to the subject.

5 Pharmaceutically acceptable carrier preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and
10 buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. The active therapeutic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include water, saline, dextrose, glycerol and ethanol, or combinations thereof. Intravenous vehicles include fluid and
15 nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

20 In addition, the therapeutic composition may further include an effective amount of one or more of the following active ingredients: at least one antibiotic, a steroid, an anti-TNF antibody and a TNF antagonist.

25 A polypeptide or antibody of the invention can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. These include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acid, or organic acids such as acetic, oxalic, tartaric and the like. Salts also include those formed from inorganic bases such as, for example, sodium, potassium,

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ammonium, calcium or ferric hydroxides, and organic bases such as isopropylamine, trimethylamine, histidine, procaine and the like.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art 5 may alternatively be used.

EXAMPLE 1
PRODUCTION OF LIPOPOLYSACCHARIDE (LPS)
BINDING POLYPEPTIDE

10 The cDNA for the amino-terminal half of human LBP was generated from full-length human LBP cDNA (Schumann, *et al.*, *Science*, 249:1429-1431, 1990) using polymerase chain reaction (PCR). Briefly, oligonucleotide primers with the sequences GTTCTAGACTGCACTGGGAATCTA (SEQ. ID NO:3) and 15 AGGAATTCAAATCTCTGTTGTAAC TG (SEQ. ID NO:4) were used. DNA polymerase was used for 20 cycles (94°C, 55°C, and 72°C for 1 min each) in an automated temperature cycler. The band corresponding to the half-molecule of LBP was purified by gel electrophoresis and ligated into the pEE14 vector (Bebbington and Hentschel, 1987) with *Xba*I and *Eco*RI sites. Analysis of the resultant DNA by restriction mapping (*Eco*RI, *Xba*I, *Bam*HI, *Cl*I, and *Nar*I) yielded fragments of the expected size.

20 CHO-K1 cells were transfected with this construct using calcium phosphate precipitation (Sambrook, *et al.*, *Molecular Cloning*, pp. 16.01-16.81, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Cells expressing the truncated LBP, hereafter referred to as NH-LBP, were selected using methionine sulfoximine at 50 μ m for 2 weeks, followed by increasing methionine sulfoximine to 200 μ m for 2 months (Bebbington and Hentschel, *DNA Cloning*, Vol III, 163-188, 1987, IRL Press, 25 Washington, D.C.).

Purification of NH-LBP was accomplished by ion exchange chromatography on Bio-Rex 70 and PL-Sax (in place of Mono Q) resins as described for human LBP (Schumann, *et al., supra*). The progress of the purification was monitored using Western blotting with polyclonal goat anti-human LBP as the detecting reagent followed by peroxidase-conjugated rabbit anti-goat IgG. The polyclonal goat anti-human LBP was prepared by immunization of a goat with human LBP expressed in an SF-9/baculovirus system (Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, pp 1-57, 1988). A recombinant baculovirus containing the coding sequence for human LBP (Schumann, *et al., supra*) was used to infect the SF-9 cells. The expressed recombinant human LBP was purified as described above for NH-LBP.

Serum-free culture supernatants from CHO cells transfected with the NH-LBP construct showed evidence of NH-LBP expression by virtue of exhibiting an extra band in SDS-PAGE which stained positively with anti-human LBP IgG in Western blotting (FIGURE 1). FIGURE 1 shows an SDS-PAGE analysis of the purification of NH-LBP. The left panel is a Coomassie-stained gel. The right panel shows a Western blot of an equivalent gel using polyclonal goat antiserum to human LBP. Lanes 1 and 5 show supernatant from untransfected cells; lanes 2 and 6 show supernatant from NH-LBP transfected cells; lanes 3 and 7 show NH-LBP partially purified by Bio-Rex 70 chromatography; and lanes 4 and 8 show human LBP.

After isolation, using immunoreactivity with anti-human LBP in Western blotting to monitor the purification, the putative NH-LBP had an apparent molecular weight of 27,000 by SDS-PAGE, in reasonable agreement with the value of 21,660 calculated from the nucleic acid sequence of the cDNA. Amino-terminal microsequencing yielded ANPGL (SEQ ID NO:5) for the protein, in agreement with the amino-terminal sequence of human LBP. Finally, the cDNA construct yielded restriction fragments of the predicted size when digested with *Eco*RI, *Xba*I, *Bam*HI, *Clal*, and *Nar*I. Thus, the isolated expressed protein had the sequence deduced from the constructed cDNA, NH-LBP.

EXAMPLE 2LBP POLYPEPTIDE BLOCKS LPS BINDING TO CD14

The ability of NH-LBP to promote or inhibit fluorescein-labeled LPS (FITC-LPS) binding to human CD14 expressing CHO cells (hCD14-CHO) was assessed by FACS analysis. FITC-LPS was prepared from *Salmonella minnesota* Re595 LPS (Galanos, *et al.*, *Eur. J. Biochem.*, 2:245-249, 1969) and fluorescein isothiocyanate as described (Skelly, *et al.*, *Infect. Immun.*, 23:287-293, 1979). hCD14-CHO cells (Kirkland, *et al.*, *J. Biochem.*, 268:24818-24823, 1993) (2×10^5 /ml) were incubated with 5 ng/ml FITC-LPS for 30 min at 22°C in Hank's balanced salt solution containing 0.3% bovine serum albumin before FACS analysis. Rabbit LBP (Tobias, *et al.*, *J. Exp. Med.* 164:77, 1986) or NH-LBP were added prior to addition of FITC-LPS. Quantitative estimation of the relative affinities of NH-LBP and LBP for FITC-LPS was accomplished as follows. From the definitions of the dissociation constants for LPS-NH-LBP and LPS-LBP complex formation and the fact that LPS is common to the two reactions one may write

$K_{NH}[\text{LPS:NH-LBP}]/[\text{NH-LBP}] = K_{LBP}[\text{LPS:LBP}]/[\text{LBP}]$. When $[\text{LPS:NH-LBP}] = [\text{LPS:LBP}]$, which occurs when NH-LBP inhibits 50% of the binding of FITC-LPS to hCD14-CHO cells, then $K_{NH}/K_{LBP} = [\text{NH-LBP}]/[\text{LBP}]$.

Direct evidence for the interaction of LPS with NH-LPS with NH-LBP was assessed with the use of ^{125}I -ASD-LPS (ASD = 2 - (p-azidosalicylamido) ethyl -1,3'-dithiopropionate). ^{125}I -ASD-LPS was prepared and photolyzed as previously described (Tobias, *et al.*, *supra*, 1986). Aliquots of each reaction mixture were analyzed on 12% SDS-PAGE, revealing the ^{125}I -labeled proteins by autoradiography. Labeled bands were identified by comparison of their mobilities with purified NH-LBP, LBP, or sCD14.

In a direct test of NH-LBP binding to LPS, NH-LBP was exposed to ^{125}I -ASD-LPS, a photoactivatable derivative of LPS which, upon photolysis, radioiodinates proteins to which it binds (Wollenweber and Morrison, *J. Biol. Chem.*, 260:15068, 1985): FIGURE 2 shows a functional analysis of NH-LBP using ^{125}I -ASD-LPS labeling. Reaction

mixtures contained LBP at 0.5×10^{-8} M and/or sCD14 at 9×10^{-8} M as indicated by (+). The concentrations of NH-LBP indicated in the figure are in units of 10^{-8} M. For lanes 1-9, all components were mixed with the 125 I-ASD-LPS added last. For lanes 10-12, the 125 I-ASD-LPS and NH-LBP were incubated for 10 min at room temperature before 5 addition of the LBP and sCD14. The complete reaction mixtures were incubated for 5 min at room temperature before photolysis for 2 min on ice.

When NH-LBP was incubated with 125 I-ASD-LPS, photolyzed, and subjected to SDS-PAGE, the 125 I band revealed by autoradiography had an apparent molecular weight of 27,000 and co-migrated with NH-LBP in the same gel as revealed by Coomassie Blue staining (FIGURE 2, lanes 2 and 3). As shown in FIGURE 2 (lane 1) and elsewhere (Schumann, *et al.*, *supra*, 1990), LBP behaves similarly toward 125 I-ASD-LPS. Thus, like LBP, NH-LBP is capable of binding 125 I-ASD-LPS.

Although NH-LBP is capable of binding LPS, it is unable to promote the binding of LPS to either sCD14 or mCD14. Several different experiments shown in FIGURES 2 and 3 support this conclusion. When LBP and sCD14 are co-incubated with 125 I-ASD-LPS for 5 min at room temperature, both the LBP and sCD14 become labeled (FIGURE 2, lane 4), although in the absence of LBP, sCD14 is not labeled in this time period. However, in mixtures of NH-LBP and sCD14 with 125 I-ASD-LPS, only NH-LBP becomes labeled, even when NH-LBP is present at 10 times the LBP concentration which leads to sCD14 labeling (FIGURE 2, lanes 5 and 6). Thus, NH-LBP does not enable 125 I-ASD-LPS to bind to sCD14.

Studies were also done to determine whether NH-LBP could successfully compete with LBP for 125 I-ASD-LPS and inhibit labeling of sCD14. When NH-LBP and LBP were co-incubated with 125 I-ASD-LPS before addition of LBP and sCD14, NH-LBP was able to inhibit 125 I-ASD-LPS labeling of sCD14, as seen by comparing lanes 6-12 with lane 4 of FIGURE 2. Preincubation of NH-LBP with 125 I-ASD-LPS was more inhibitory than co-incubation of NH-LBP and LBP with 125 I-ASD-LPS (compare FIGURE 2, lanes 10-12

with 7-9). Judging by the slightly greater intensity of the radiolabeled NH-LBP than the radiolabeled LBP in lane 9 (FIGURE 2), one might estimate that the affinity of NH-LBP for ^{125}I -ASD-LPS is 50-100 fold less than the affinity of LBP for ^{125}I -ASD-LPS. The affinity of LBP for Re595 LPS, used to prepare ^{125}I -ASD-LPS, is estimated at 1×10^{-9} M (Tobias, *et al.*, *J. Biol. Chem.* 264:10867, 1989).

The aforementioned properties of NH-LBP are not limited to ^{125}I -ASD-LPS binding to sCD14 but can also be seen with membrane-bound CD14. CHO cells were transfected with a plasmid bearing the cDNA for human mCD14 and characterized the resulting cells (hCD14-CHO) as expressing a glycerophosphorylinositol-bound form of human CD14 (Kirkland, *et al.*, *supra*, 1993). The binding of [^3H]LPS to these cells has recently been described (Kirkland, *et al.*, *supra*, 1993). FIGURE 3 shows A, FACS analysis of FITC-LPS binding to hCD14-CHO cells. Peak 1 shows cells alone; 2, FITC-LPS plus NH-LBP with cells; 3, FITC-LPS plus LBP with cells; 4, FITC-LPS plus NH-LBP plus LBP with cells. FIGURE 3B shows inhibition of FITC-LPS binding to hCD14-CHO cells by NH-LBP. hCD14-CHO cells were mixed with FITC-LPS (5 ng/ml) in the presence of the indicated concentrations of NH-LBP with (□) or without (○) LBP (100 ng/ml).

When incubated with FITC-LPS (FIGURE 3A), the hCD14-CHO cells do not bind FITC-LPS unless LBP is added. In this regard, the hCD14-CHO cells display FITC-LPS binding that resembles the binding displayed by peripheral blood monocytes (Heumann, *et al.*, *J. Immunol.*, 148:3505-3512, 1992). Nh-LBP is unable to substitute for LBP and inhibits LBP-assisted FITC-LPS binding (FIGURE 3A). The dose dependency of NH-LBP inhibition is shown in FIGURE 3B. The binding of FITC-LPS to the cells enabled by 100 ng/ml LBP (1.7×10^{-9} M) is 50% inhibited by 250 ng/ml NH-LBP (1.1×10^{-8} M), suggesting that the dissociation constant of NH-LBP-LPS complexes is 6.4-fold larger (i.e., weaker binding) than the dissociation constant for LBP-LPS complexes, estimated to be 1×10^{-9} M (Tobias, *et al.*, 1989). The FACS measurement of FITC-LPS binding to hCD14-CHO cells is more quantitative than the densities of the autoradiogram in FIGURE 2, therefore one might give greater weight to this latter estimate of the relative

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affinities of LBP and NH-LBP for LPS. However, whichever estimate for the affinity of NH-LBP for LPS one accepts, the data suggest that the NH-LBP fragment must retain most of the LPS binding site.

EXAMPLE 3

5 **ACTIVATION OF MACROPHAGES BY LBP POLYPEPTIDE**

The effect of NH-LBP on macrophage activation was studied using rabbit peritoneal exudate macrophages (PEM). Mineral oil-elicited rabbit PEM were cultured in serum-free medium as described previously (Mathison, *et al.*, *J. Clin. Invest.*, 81:1925-1937, 1988). PEM were cultured in 96-well clusters (100- μ l suspension containing 1×10^5 cells) for 2 h followed by washing to remove nonadherent cells and replenishment with 50 μ l of serum-free medium. To minimize binding of LBP to the polystyrene wells, the culture medium was supplemented with 1% human serum albumin (Miles, Inc., Cutter Biological, Lot 88G04). NH-LBP (1 μ g/ml final concentration) was added to macrophages followed immediately by native purified rabbit LBP (10 ng/ml final concentration) and 1 ng/ml 0111:B4 LPS. After 4 h at 37°C, 5% CO₂, conditioned medium was harvested for assay of tumor necrosis factor cytolytic activity using L929 cells (Mathison, *et al.*, *supra*, 1988).

20 The aforementioned prompted an examination of the effects of NH-LBP on LPS-induced cell activation by the LBP/CD14-dependent pathway. To do this, the effect of NH-LBP on LPS-dependent activation of rabbit PEM to produce tumor necrosis factor was evaluated. FIGURE 4 shows the inhibition of LPS-initiated rabbit PEM activation by NH-LBP. Results are shown as TNF (Units/ml) for LBP and LBP+NH-LBP.

As shown in FIGURE 4, NH-LBP inhibited the LPS-and LBP-dependent activation of rabbit PEM.

FIGURES 5, 6 and 7 show the nucleotide and deduced amino acid sequence of human LBP, amino acid residues 1-197, and 198-481 of LBP, respectively.

The two functions of LBP, binding LPS and promoting the formation of LPS:CD14 complexes, are therefore, two distinct functions mediated by structurally distinct moieties 5 of LBP. Clearly, the LPS binding function resides largely if not entirely within the NH-LBP fragment. In this regard, the closely related LPS binding proteins LBP and BPI resemble each other since the LPS binding site of BPI has been localized to sequences contained within a 23-kDa truncated form of BPI specifying residues 1-200 (Ooi, *et al.*, *J. Exp. Med.*, 174:649-655, 1991). However, these studies shown in the Examples point 10 out a marked difference between the functional domains of LBP and BPI since the 23-kDa form of BPI not only binds LPS but also defines the region of the molecule responsible for its antibacterial properties. Cholesteryl ester transfer protein, a protein that shows 15 amino acid sequence similarities with both LBP and BPI (Schumann, *et al.*, *supra*, 1990), also appears to have two distinct functional domains. However, the domains specifying the binding sites for cholesteryl esters and phospholipids (Swenson, *et al.*, *J. Biol. Chem.* 263:5150, 1988) have not yet been associated with physically distinct entities.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope 20 of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE SCRIPPS RESEARCH INSTITUTE

5 (ii) TITLE OF INVENTION: POLYPEPTIDES OF LIPOPOLYSACCHARIDE
BINDING PROTEIN

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

10 (A) ADDRESSEE: SPENSLEY HORN JUBAS & LUBITZ
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(D) STATE: CALIFORNIA
(E) COUNTRY: US
(F) ZIP: 90067

15 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 15-MAR-1995
(C) CLASSIFICATION:

25 (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HAILE, PH.D., LISA A.
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(C) REFERENCE/DOCKET NUMBER: FD-3372

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30 (A) TELEPHONE: 619/455-5100
(B) TELEFAX: 619/455-5110

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 620 base pairs
- (B) TYPE: nucleic acid
- 5 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: N-terminal LBP

10 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 30..620

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

15	CTCCTGGCCC ACTGCACTGG GAATCTAGG ATG GGG GCC TTG GCA AGA GCC CTG Met Gly Ala Leu Ala Arg Ala Leu 1 5	53
20	CCG TCC ATA CTC CTC GCA TTG CTG CTT ACC TCC ACC CCA GAG GCT CTC Pro Ser Ile Leu Leu Ala Leu Leu Leu Thr Ser Thr Pro Glu Ala Leu 10 15 20	101
25	GGT GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG Gly Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln 25 30 35 40	149
30	TAT GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG Tyr Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg 45 50 55	197
35	ATC ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC Ile Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly 60 65 70	245
40	CGT GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG Arg Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu 75 80 85	293

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	CTT CAC TCT GCG CTG AGG CCT GTC CCC GGC CAG GGC CTG AGT CTC AGC	341		
	Leu His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser			
	90	95	100	
5	ATC TCC GAC TCC TCC ATC CGG GTC CAG GGC AGG TGG AAG GTC CGC AAG	389		
	Ile Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys			
	105	110	115	120
	TCA TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC	437		
	Ser Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile			
	125	130	135	
10	AGC ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGG AGG CCC	485		
	Ser Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro			
	140	145	150	
15	ACA GGT TAC TGC CTC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG	533		
	Thr Gly Tyr Cys Leu Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val			
	155	160	165	
	GAC ATG TCC GGA GAT TCG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG	581		
	Asp Met Ser Gly Asp Ser Gly Trp Leu Leu Asn Leu Phe His Asn Gln			
	170	175	180	
20	ATT GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT	620		
	Ile Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile			
	185	190	195	

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 197 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu			
30	1	5	10	15
	Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala			
	20	25	30	

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Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
35 40 45

Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
50 55 60

5 Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
65 70 75 80

Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
85 90 95

Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
10 100 105 110

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
115 120 125

Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
130 135 140

15 Gly Ser Glu Ser Ser Gly Arg Pro Thr Gly Tyr Cys Leu Ser Cys Ser
145 150 155 160

Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Ser Gly Trp
165 170 175

Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
20 180 185 190

Leu Glu Ser Arg Ile
195

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

5 GTTCTAGACT GCACTGGGAA TCTA

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..26

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGGAATTCAA ATCTCTGTTG TAACTG

26

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

25

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..5

-34-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Asn Pro Gly Leu
 1 5

(2) INFORMATION FOR SEQ ID NO:6:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 852 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
 (B) CLONE: C-terminal LBP

15 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..852

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGC GAA ATG ATC CAG AAA TCA GTG TCC TCC GAT CTA CAG CCT TAT CTC	48
Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu	
1 5 10 15	
20 CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT TTC GCC GAC ATT GAT	96
Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser Phe Ala Asp Ile Asp	
20 25 30	
25 TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC CAG ATG CTG GAG GTG	144
Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala Gln Met Leu Glu Val	
25 35 40 45	
30 ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC CAG TCT CCA GTT ACC	192
Met Phe Lys Gly Glu Ile Phe His Arg Asn His Arg Ser Pro Val Thr	
50 55 60	
30 CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA CAC AAC AAA ATG GTC	240
Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu His Asn Lys Met Val	
65 70 75 80	

-35-

	TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG GCC AGC CTG GTT TAT Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr Ala Ser Leu Val Tyr 85 90 95	288
5	CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA GAT GAC ATG ATA CCG His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr Asp Asp Met Ile Pro 100 105 110	336
	CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC TTC CGA CCC TTC GTC Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser Phe Arg Pro Phe Val 115 120 125	384
10	CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC CTG GAA CTC CAG GGA Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn Leu Glu Leu Gln Gly 130 135 140	432
15	TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC CCT GGG AAT CTG TCT Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser Pro Gly Asn Leu Ser 145 150 155 160	480
	GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG CTC CTG CCC AGC TCC Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val Leu Leu Pro Ser Ser 165 170 175	528
20	AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC ACT AAT GTG TCC GCC Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala Thr Asn Val Ser Ala 180 185 190	576
	ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG TTC CTG AAG CCA GGA Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly Phe Leu Lys Pro Gly 195 200 205	624
25	AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT GGA CTA TTC AAT GCA Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val Gly Leu Phe Asn Ala 210 215 220	672
	GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC CTT AAC ACC CTC TAC Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile Leu Asn Thr Leu Tyr 225 230 235 240	720
30	CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC CCC CTT CCT CTG CTG Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe Pro Leu Pro Leu Leu 245 250 255	768

-37-

Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn Leu Glu Leu Gln Gly
130 135 140

Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser Pro Gly Asn Leu Ser
145 150 155 160

5 Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val Leu Leu Pro Ser Ser
165 170 175

Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala Thr Asn Val Ser Ala
180 185 190

Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly Phe Leu Lys Pro Gly
10 195 200 205

Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val Gly Leu Phe Asn Ala
210 215 220

Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile Leu Asn Thr Leu Tyr
225 230 235 240

15 Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe Pro Leu Pro Leu Leu
245 250 255

Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln Ile His Lys Asp Phe
260 265 270

Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg Val
20 275 280

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1801 base pairs
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: human LBP

-38-

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1443

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

5	ATG GGG GCC TTG GCA AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu 1 5 10 15	48
10	CTT ACG TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala 20 25 30	96
15	AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu 35 40 45	144
20	GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly 50 55 60	192
25	GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC AGC Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser 65 70 75 80	240
30	CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val 85 90 95	288
35	CCC GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val 100 105 110	336
40	CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser 115 120 125	384
45	TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu 130 135 140	432

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	GGC AGC GAG TCC TCC GGG AGG CCC ACA GGT TAC TGC CTC AGC TGC AGC		480
	Gly Ser Glu Ser Ser Gly Arg Pro Thr Gly Tyr Cys Leu Ser Cys Ser		
	145 150 155 160		
5	AGT GAC ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAT TCG GGG TGG		528
	Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Ser Gly Trp		
	165 170 175		
	CTC TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA		576
	Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val		
	180 185 190		
10	CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCA GTG TCC TCC GAT		624
	Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp		
	195 200 205		
15	CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT		672
	Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser		
	210 215 220		
	TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC		720
	Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala		
	225 230 235 240		
20	CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC		768
	Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His		
	245 250 255		
	CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA		816
	Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu		
	260 265 270		
25	CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG		864
	His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr		
	275 280 285		
30	GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA		912
	Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr		
	290 295 300		
	GAT GAC ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC		960
	Asp Asp Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser		
	305 310 315 320		

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	TTC CGA CCC TTC GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC	1008		
	Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn			
	325	330	335	
	CTG GAA CTC CAG GGA TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC	1056		
5	Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser			
	340	345	350	
	CCT GGG AAT CTG TCT GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG	1104		
	Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val			
	355	360	365	
10	CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC	1152		
	Leu Leu Pro Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala			
	370	375	380	
	ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG	1200		
15	Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly			
	385	390	395	400
	TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT	1248		
	Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val			
	405	410	415	
20	GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC	1296		
	Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile			
	420	425	430	
	CTT AAC ACC CTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC	1344		
	Leu Asn Thr Leu Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe			
	435	440	445	
25	CCC CTT CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG	1392		
	Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln			
	450	455	460	
	ATC CAT AAG GAC TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA	1440		
	Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg			
30	465	470	475	480
	GTT TGAGGACAAG AAAGATGAAG CTTGGAGGTC ACAGGGCTGGA TCTGCTTGTT	1493		
	Val			
	GCATTTCCAG CTGTGCAGCA CGTCTCAGAG ATTCTTGAAG AATGAAGACA TTTCTGCTCT	1553		

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Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
370 375 380

Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
385 390 395 400

5 Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
405 410 415

Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
420 425 430

Leu Asn Thr Leu Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
10 435 440 445

Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
450 455 460

Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
465 470 475 480

15 Val

CLAIMS

1. An isolated lipopolysaccharide (LPS) binding polypeptide with an amino acid sequence of SEQ ID NO:2 and functional fragments thereof or with the amino acid sequence of SEQ ID NO:7 and functional fragments thereof.
2. An isolated polynucleotide which encodes the polypeptide of LPS binding protein of SEQ ID NO:2 or SEQ ID NO:7.
3. The polynucleotide of claim 2, wherein the LPS binding protein nucleotide sequence is selected from the group consisting of the nucleic acid sequence of
 - a. SEQ ID NO:1, wherein T can also be U;
 - b. SEQ ID NO:6, wherein T can also be U;
 - 5 c. nucleic acid sequences complementary to SEQ ID NO:1;
 - d. nucleic acid sequences complementary to SEQ ID NO:6;
 - e. fragments of a. or c. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes polypeptide of LPS binding protein of SEQ ID NO:2.
 - f. fragments of b. or d. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes polypeptide of LPS binding protein of SEQ ID NO:7.
4. An antibody which binds to the amino acid sequence of SEQ ID NO:2 or to the amino acid sequence of SEQ ID NO:7.
5. The antibody of claim 4, wherein the antibody is polyclonal.
6. The antibody of claim 5, wherein the antibody is monoclonal.

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Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
370 375 380

Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
385 390 395 400

5 Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
405 410 415

Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
420 425 430

Leu Asn Thr Leu Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
10 435 440 445

Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
450 455 460

Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
465 470 475 480

15 Val

CLAIMS

1. An isolated lipopolysaccharide (LPS) binding polypeptide with an amino acid sequence of SEQ ID NO:2 and functional fragments thereof or with the amino acid sequence of SEQ ID NO:7 and functional fragments thereof.
2. An isolated polynucleotide which encodes the polypeptide of LPS binding protein of SEQ ID NO:2 or SEQ ID NO:7.
3. The polynucleotide of claim 2, wherein the LPS binding protein nucleotide sequence is selected from the group consisting of the nucleic acid sequence of
 - a. SEQ ID NO:1, wherein T can also be U;
 - b. SEQ ID NO:6, wherein T can also be U;
 - 5 c. nucleic acid sequences complementary to SEQ ID NO:1;
 - d. nucleic acid sequences complementary to SEQ ID NO:6;
 - e. fragments of a. or c. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes polypeptide of LPS binding protein of SEQ ID NO:2.
 - f. fragments of b. or d. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes polypeptide of LPS binding protein of SEQ ID NO:7.
4. An antibody which binds to the amino acid sequence of SEQ ID NO:2 or to the amino acid sequence of SEQ ID NO:7.
5. The antibody of claim 4, wherein the antibody is polyclonal.
6. The antibody of claim 5, wherein the antibody is monoclonal.

7. The antibody of claim 6, wherein the antibody competitively inhibits the binding of LPS binding protein (LBP) to LPS.
8. The antibody of claim 6, wherein the antibody competitively inhibits the binding of LPS binding protein:LPS to CD14.
9. A method of detecting lipopolysaccharide (LPS) endotoxin in a sample from a mammal comprising:
 - a. contacting a sample of body fluid suspected of containing LPS with a polypeptide of LPS binding protein wherein the polypeptide forms a complex with LPS which does not bind to CD14 receptor;
 - b. incubating the mixture for a sufficient time to allow the LPS and the polypeptide of LPS-binding protein to bind; and
 - c. detecting the LPS of LPS-binding protein complex.
10. The method of claim 9, wherein the polypeptide of LPS-binding protein is the amino acid sequence of SEQ ID NO:2.
11. The method of claim 9, wherein the LPS is derived from a gram-negative bacteria.
12. The method of claim 9, wherein the detection is *in vitro*.
13. The method of claim 9, wherein the detection is *in vivo*.
14. The method of claim 9, wherein the polypeptide of LPS-binding protein is labeled.

15. The method of claim 14, wherein the label is selected from the group consisting of enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds.
16. A method of ameliorating sepsis comprising administering to a subject with symptoms of sepsis or at risk for developing sepsis, a therapeutically effective amount of:
 - a. a polypeptide of LPS-binding protein wherein the polypeptide forms a complex with LPS which does not bind to CD14 receptor;
 - b. a polypeptide of LPS-binding protein wherein the polypeptide is inhibits the binding of LPS:LBP complex to CD14 receptor via interaction with the CD14 receptor;
 - c. antibody to a. or b.; and
 - d. mixtures of a., b., and c.
- 5
17. The method of claim 16, wherein the polypeptide of LPS binding protein is the polypeptide of SEQ ID NO:2.
18. The method of claim 16, wherein the antibody binds to the polypeptide of SEQ ID NO:2.
19. The method of claim 16, wherein the polypeptide of LPS binding protein is the polypeptide of SEQ ID NO:7.
20. The method of claim 16, wherein the antibody binds to the polypeptide of SEQ ID NO:7.
21. The method of claim 18, wherein the antibody is monoclonal.

22. The method of claim 21, wherein the antibody inhibits the binding of LPS binding protein to LPS.
23. The method of claim 20, wherein the antibody is monoclonal.
24. The method of claim 23, wherein the antibody inhibits the binding of LPS:LBP complex to CD14 receptor.
25. The method of claim 16, wherein the antibody is an anti-idiotype antibody.
26. The method of claim 25, wherein the anti-idiotype antibody binds to a paratope of an antibody which binds to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:7.
27. The method of claim 16, further comprising administering to the subject a bactericidal amount of antibiotic.
28. The method of claim 16, further comprising administering to the subject a tumor necrosis factor (TNF)-blood concentration reducing amount of anti-TNF antibody.
29. The method of claim 27, further comprising administering to the subject a TNF-blood concentration reducing amount of anti-TNF antibody.
30. The method of claim 27, wherein the antibiotic is an anti-bacterial agent effective against gram-negative bacteria.
31. The method of claim 16, wherein the sepsis is caused by a gram- negative bacterial infection, a virus, a gram-positive bacterial infection or a fungus.

32. The method of claim 16, wherein the symptoms of sepsis include one or more of the following: adult respiratory distress syndrome, disseminated intravascular coagulation, renal failure and hepatic failure.
33. A method of ameliorating sepsis in a subject comprising administering to a subject with symptoms of sepsis or at risk for developing sepsis an effective amount of polypeptide of LPS binding protein or antibody to polypeptide of LPS binding protein sufficient to inhibit LPS-induced TNF secretion by a myeloid cell in the subject.
34. The method of claim 33, wherein the LPS binding protein is the polypeptide of SEQ ID NO:2 or SEQ ID NO:7.
35. The method of claim 33, wherein the antibody binds to the polypeptide of SEQ ID NO:2 or SEQ ID NO:7.
36. The method of claim 35, wherein the antibody is monoclonal.
37. The method of claim 36, wherein the antibody competitively inhibits the binding of LPS binding protein to LPS or LPS:LPS binding protein complex to CD14.
38. The method of claim 33, wherein the antibody is an anti-idiotype antibody.
39. The method of claim 38, wherein the anti-idiotype antibody binds to a paratope of an antibody which binds to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:7.
40. The method of claim 33, further comprising administering to the subject a bactericidal amount of antibiotic.

41. The method of claim 33, further comprising administering to the subject a tumor necrosis factor (TNF)-blood concentration reducing amount of anti-TNF antibody.
42. The method of claim 40, further comprising administering to the subject a TNF-blood concentration reducing amount of anti-TNF antibody.
43. The method of claim 40, wherein the antibiotic is an anti-bacterial agent effective against gram-negative bacteria.
44. The method of claim 33, wherein the sepsis is caused by a gram- negative bacterial infection, a virus, a gram-positive bacterial infection or a fungus.
45. The method of claim 33, wherein the symptoms of sepsis include one or more of the following: adult respiratory distress syndrome, disseminated intravascular coagulation, renal failure and hepatic failure.
46. A therapeutic pharmaceutical composition comprising a polypeptide of LPS-binding protein which inhibits the binding of an LPS:LBP complex to CD14 in a pharmaceutical carrier.
47. The composition of claim 46, wherein the LPS-binding protein polypeptide has the amino acid sequence of SEQ ID NO:2.
48. The composition of claim 46, further comprising anti-TNF antibody.
49. The composition of claim 46, further comprising a bactericidal amount of an antibiotic.
50. The composition of claim 48, further comprising a bactericidal amount of an antibiotic.

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51. The composition of claim 46, wherein the LPS-binding protein polypeptide has the amino acid sequence of SEQ ID NO:7.

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1 2 3 4

5 6 7 8

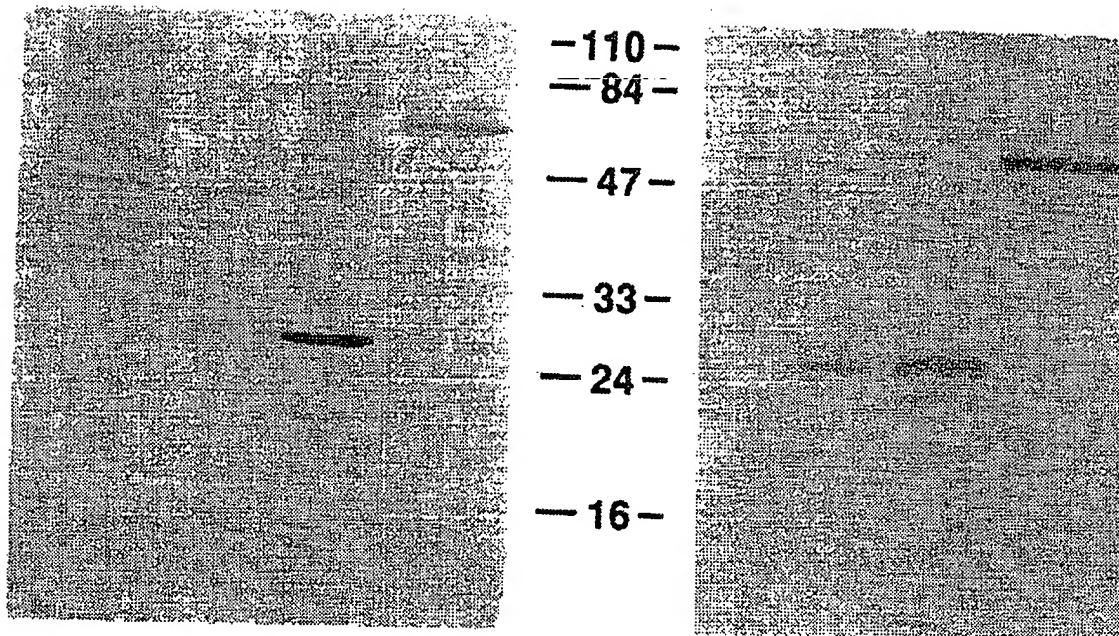


FIG. 1

2/10

Lane #	1	2	3	4	5	6	7	8	9	10	11	12
LBP	+	-	-	+	-	-	+	+	+	+	+	+
sCD14	-	-	-	+	+	+	+	+	+	+	+	+
NH-LBP	0	0.5	5	0	0.5	5	0.5	5	50	0.5	5	50

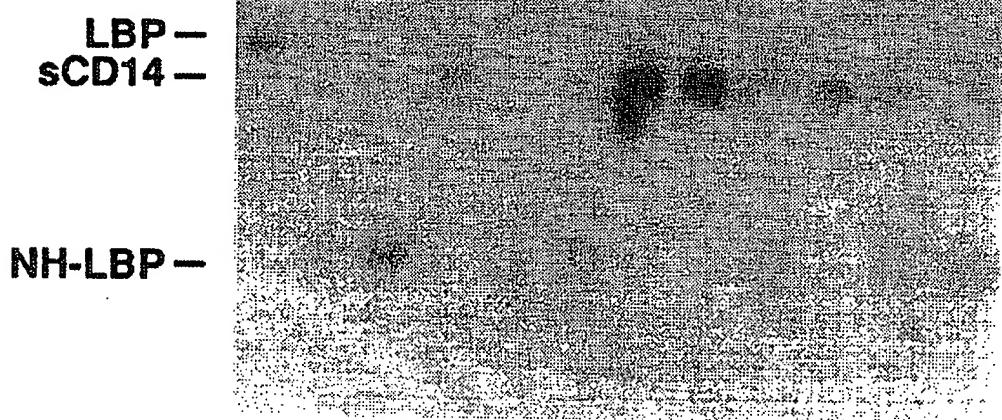


FIG. 2

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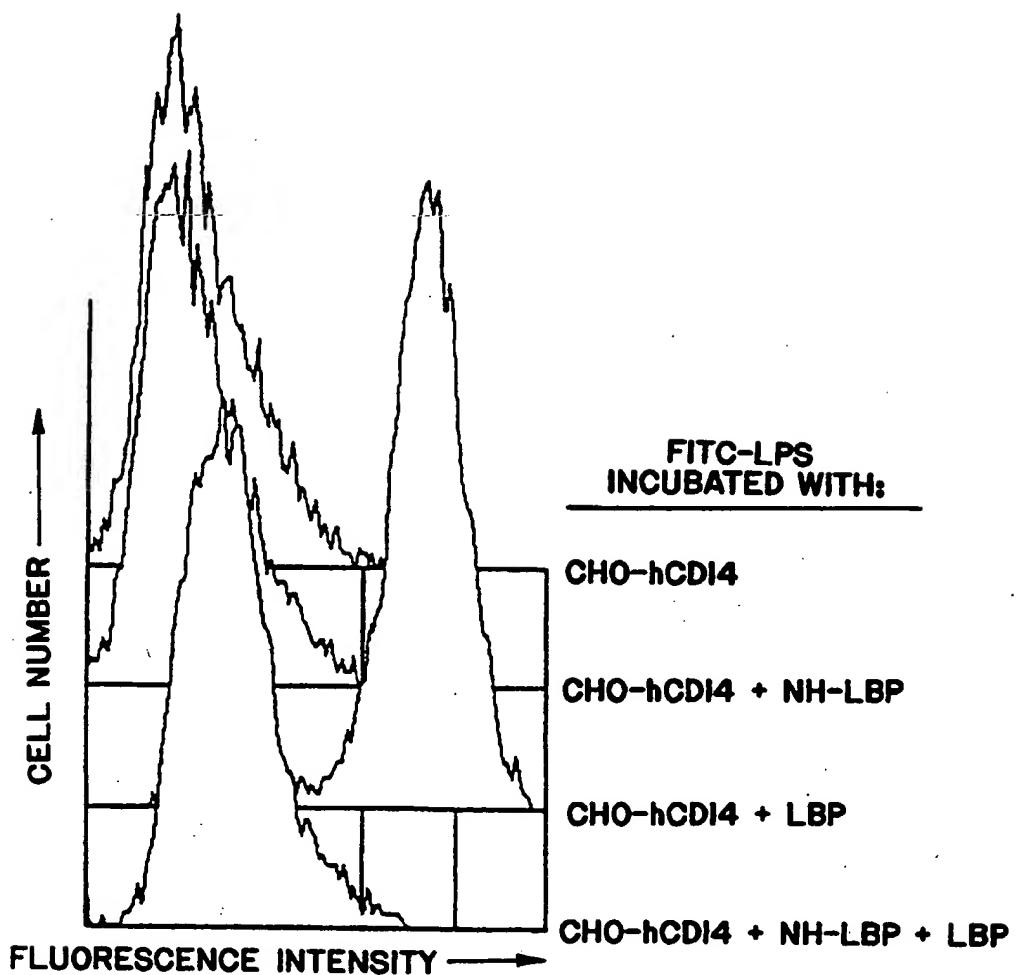


FIG. 3A

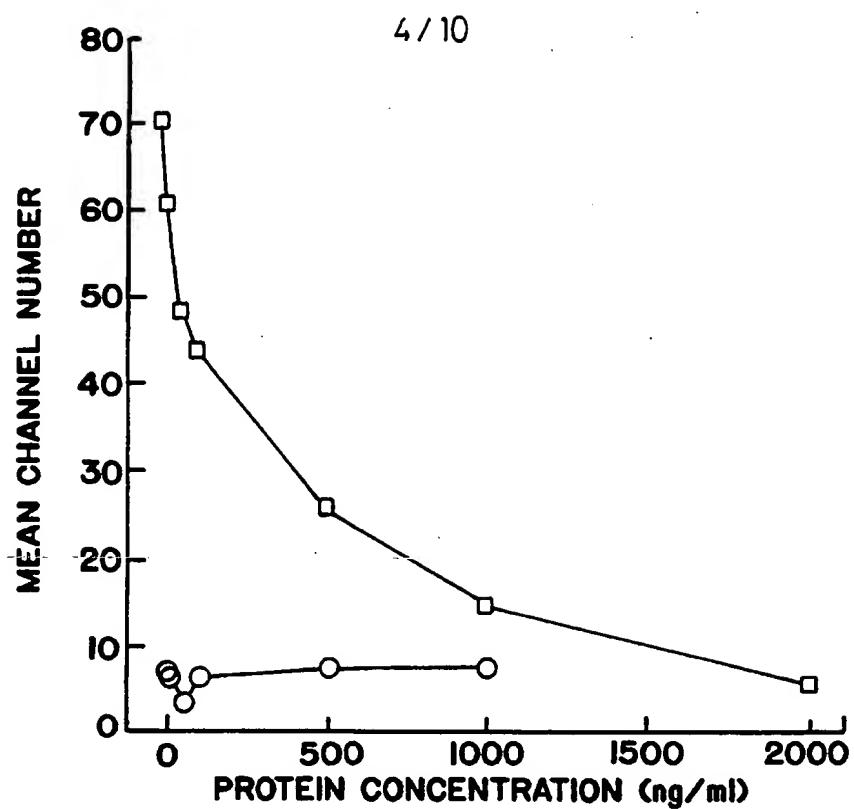


FIG. 3B

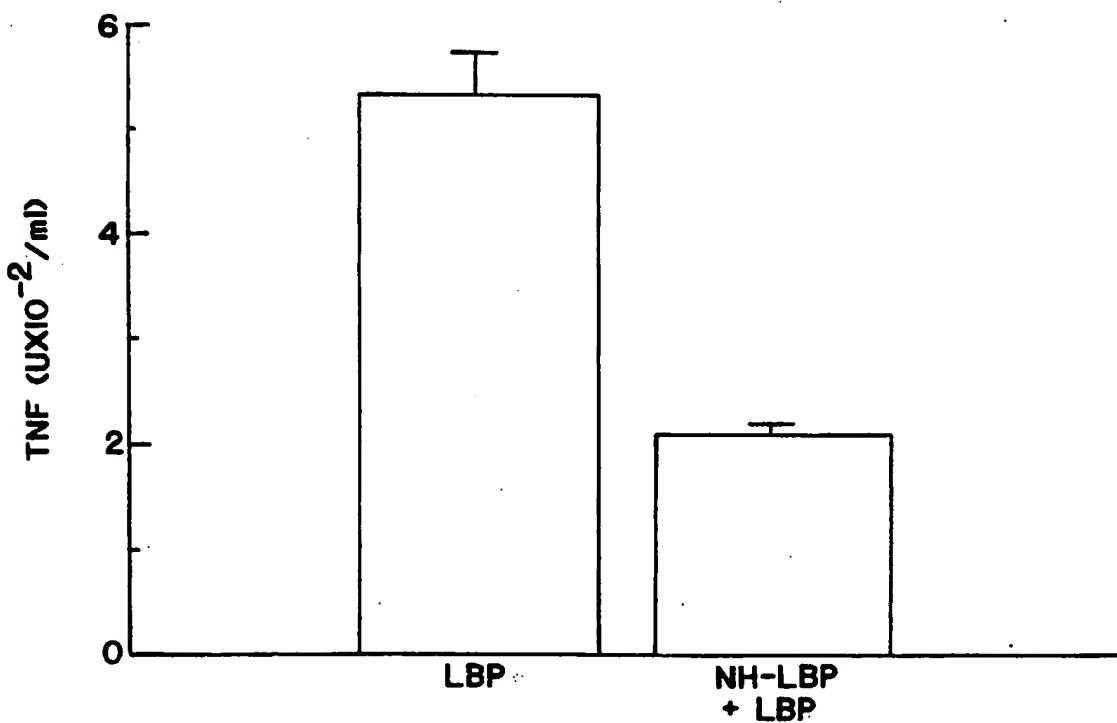


FIG. 4

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ATG GGG GCC TTG GCA AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG	48
Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu	
1 5 10 15	
CTT ACG TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC	96
Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala	
20 25 30	
AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG	144
Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu	
35 40 45	
GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG	192
Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly	
50 55 60	
GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC AGC	240
Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser	
65 70 75 80	
CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC	288
Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val	
85 90 95	
CCC GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC	336
Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val	
100 105 110	
CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC AAA CTA CAG GGC TCC	384
Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser	
115 120 125	
TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG	432
Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu	
130 135 140	
GGC AGC GAG TCC TCC GGG AGG CCC ACA GGT TAC TGC CTC AGC TGC AGC	480
Gly Ser Glu Ser Ser Gly Arg Pro Thr Gly Tyr Cys Leu Ser Cys Ser	
145 150 155 160	
AGT GAC ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAT TCG GGG TGG	528
Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Ser Gly Trp	
165 170 175	
CTC TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA	576
Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val	
180 185 190	
CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCA GTG TCC TCC GAT	624
Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp	
195 200 205	

FIG. 5A

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CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT	672
Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser	
210 215 220	
TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC	720
Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala	
225 230 235 240	
CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC	768
Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His	
245 250 255	
CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA	816
Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu	
260 265 270	
CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG	864
His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr	
275 280 285	
GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA	912
Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr	
290 295 300	
GAT GAC ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC	960
Asp Asp Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser	
305 310 315 320	
TTC CGA CCC TTC GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC	1008
Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn	
325 330 335	
CTG GAA CTC CAG GGA TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC	1056
Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser	
340 345 350	
CCT GGG AAT CTG TCT GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG	1104
Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val	
355 360 365	
CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC	1152
Leu Leu Pro Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala	
370 375 380	
ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG	1200
Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly	
385 390 395 400	
TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT	1248
Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val	
405 410 415	

FIG. 5B

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GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC	1296
Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile	
420 425 430	
CTT AAC ACC CTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC	1344
Leu Asn Thr Leu Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe	
435 440 445	
CCC CTT CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG	1392
Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln	
450 455 460	
ATC CAT AAG GAC TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA	1440
Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg	
465 470 475 480	
GTT TGAGGACAAG AAAGATGAAG CTTGGAGGTC ACAGGGCTGGA TCTGCTTGTT	1493
Val	
GCATTTCCAG CTGTGCAGCA CGTCTCAGAG ATTCTTGAAG AATGAAGACA TTTCTGCTCT	1553
CAGCTCCGGG GGTGAGGTGT GCCTGGCCTC TGCCTCCACC CTCCCTCTCT TCACCCAGGTG	1613
CATGCATGCC CTCTCTGAGT CTGGACTTTG CTTCCCTCC AGGAGGGACC ACCCTCCCCG	1673
ACTGGCCTGG GATATCTTA CAAGCAGGCA CTGTATTTT TTATTGCGCCA TCTGATCCCC	1733
ATGCCTAGCA GAGTGCTGGC ACTTAGTAGG TCCTCAATAA ATATTTAGGT CGACGAGCTC	1793
GAGAATTC	1801

FIG. 5C

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CTCCTGGCCC ACTGCAGTGG GAATCTAGG ATG GGG GCC TTG GCA AGA GCC CTG Met Gly Ala Leu Ala Arg Ala Leu	53
1 5	
CCG TCC ATA CTC CTC GCA TTG CTG CTT ACC TCC ACC CCA GAG GCT CTC Pro Ser Ile Leu Leu Ala Leu Leu Leu Thr Ser Thr Pro Glu Ala Leu	101
10 15 20	
GGT GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG Gly Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln	149
25 30 35 40	
TAT GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG Tyr Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg	197
45 50 55	
ATC ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC Ile Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly	245
60 65 70	
CGT GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG Arg Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu	293
75 80 85	
CTT CAC TCT GCG CTG AGG CCT GTC CCC GGC CAG GGC CTG AGT CTC AGC Leu His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser	341
90 95 100	
ATC TCC GAC TCC TCC ATC CCG GTC CAG GGC AGG TGG AAG GTC CGC AAG Ile Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys	389
105 110 115 120	
TCA TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC Ser Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile	437
125 130 135	
AGC ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGG AGG CCC Ser Ile Ser Val Asn Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro	485
140 145 150	
ACA GGT TAC TGC CTC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG Thr Gly Tyr Cys Leu Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val	533
155 160 165	
GAC ATG TCC GGA GAT TCG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG Asp Met Ser Gly Asp Ser Gly Trp Leu Leu Asn Leu Phe His Asn Gln	581
170 175 180	
ATT GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT Ile Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile	620
185 190 195	

FIG. 6

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TGC GAA ATG ATC CAG AAA TCA GTG TCC TCC GAT CTA CAG CCT TAT CTC	48
Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu	
1 5 10 15	
CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT TTC GCC GAC ATT GAT	96
Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser Phe Ala Asp Ile Asp	
20 25 30	
TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC CAG ATG CTG GAG GTG	144
Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala Gln Met Leu Glu Val	
35 40 45	
ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC CGT TCT CCA GTT ACC	192
Met Phe Lys Gly Glu Ile Phe His Arg Asn His Arg Ser Pro Val Thr	
50 55 60	
CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA CAC AAC AAA ATG GTC	240
Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu His Asn Lys Met Val	
65 70 75 80	
TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG GCC AGC CTG GTT TAT	288
Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr Ala Ser Leu Val Tyr	
85 90 95	
CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA GAT GAC ATG ATA CCG	336
His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr Asp Asp Met Ile Pro	
100 105 110	
CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC TTC CGA CCC TTC GTC	384
Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser Phe Arg Pro Phe Val	
115 120 125	
CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC CTG GAA CTC CAG GGA	432
Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn Leu Glu Leu Gln Gly	
130 135 140	

FIG. 7A

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TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC CCT GGG AAT CTG TCT	480
Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser Pro Gly Asn Leu Ser	
145 150 155 160	
GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG CTC CTG CCC AGC TCC	528
Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val Leu Leu Pro Ser Ser	
165 170 175	
AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC ACT AAT GTG TCC GCC	576
Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala Thr Asn Val Ser Ala	
180 185 190	
ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG TTC CTG AAG CCA GGA	624
Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly Phe Leu Lys Pro Gly	
195 200 205	
AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT GGA CTA TTC AAT GCA	672
Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val Gly Leu Phe Asn Ala	
210 215 220	
GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC CTT AAC ACC CTC TAC	720
Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile Leu Asn Thr Leu Tyr	
225 230 235 240	
CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC CCC CTT CCT CTG CTG	768
Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe Pro Leu Pro Leu Leu	
245 250 255	
AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG ATC CAT AAG GAC TTC	816
Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln Ile His Lys Asp Phe	
260 265 270	
CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA GTT	852
Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg Val	
275 280	

FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/03384

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 4/10, 16/12, 16/28; A61K 39/02
US CL :424/197.11; 530/351, 388.4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/197.11; 530/351, 388.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Sequence searched (seq. id. no. 8)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS, MEDLINE, search terms: LPS, LBP, LPS binding protein, lipopolysaccharide binding protein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Vol. 249, issued 21 September 1990, Schumann et al., "Structure and Function of Lipopolysaccharide Binding Protein ", pages 1429-1431, see entire document.	1-51
Y	Journal of Experimental Medicine, Vol. 174, issued September 1991, Ooi et al., "Endotoxin-neutralizing Properties of the 25 kD N-Terminal Fragment and a Newly Isolated 30 kD C-Terminal Fragment of the 55-60 kD Bactericidal/Permeability-increasing Protein of Human Neutrophils", pages 649-655, see entire document.	1-51

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

07 JUNE 1995

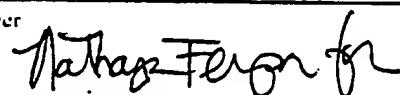
Date of mailing of the international search report

12 JUL 1995

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INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US95/03384**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Experimental Medicine, Vol. 164, issued September 1986, Tobias et al., "Isolation of a Lipopolysaccharide-Binding Acute Phase Reactant from Rabbit Serum", pages 777-793, see entire document.	1-51
Y	The Journal of Biological Chemistry, Vol. 263, No. 11, issued 15 April 1988, Swenson et al., "Plasma Cholesteryl Ester Transfer Protein Has Binding Sites for Neutral Lipids and Phospholipids", pages 5150-5157, see entire document.	1-51